



Short communication

Polyclonals to β -amyloid(1–42) identify most plaque and vascular deposits in Alzheimer cortex, but not striatumKenneth Mak ^a, Fusheng Yang ^a, Harry V. Vinters ^b, Sally A. Frautschy ^a, Gregory M. Cole ^{a,*}^a Department of Medicine, UCLA and Sepulveda VAMC, GRECC 11E, 16111 Plummer St., Sepulveda, CA 91343, USA^b Department of Pathology and Laboratory Medicine, UCLA, Los Angeles, CA, USA

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Abstract

Alzheimer's β -peptides ($A\beta$) aggregation rates depend on $A\beta$ length. We made synthetic peptide antisera to $A\beta$ 34–40 and 37–42. Purified anti-34–40 preferentially recognizes β 1–40, vascular amyloid and a subset of plaques while purified anti-37–42 recognizes $A\beta$ 1–42 and not $A\beta$ 1–40 in dot and Western blots and immunoprecipitates; 37–42 precipitates a small percentage of fibroblast secreted $A\beta$ and strongly stains all deposits identified by monoclonals to $A\beta$ on adjacent sections from frontal cortex, but not in dorsal striatum.

Keywords: Alzheimer's disease; β -Amyloid; Antibody; Cerebral cortex; Striatum

Deposition of a 28–43 amino acid peptide, β -protein ($A\beta$) accompanies Alzheimer's disease (AD) [11,12]. $A\beta$ peptides are normally secreted by cultured cells [7,14] and found in CSF [13,14]. Sequencing from cultured cell conditioned media (CM) [5,13,16] and mass spectrographic data from CSF [13] revealed C-terminal heterogeneity with β 1–40 (β 40) as the major species. In contrast, in isolated amyloid deposits, β 1–42 (β 42) appears the major species (85–95% of plaque core $A\beta$) [12]. Synthetic peptide experiments show that β 42 nucleates aggregation of $A\beta$ far better than β 39 or β 40 [2,9]. In contrast to shorter forms, β 42 tends to aggregate, is not readily cleared and accumulates in cells [10]. Collectively, these data argue that the C-terminus of $A\beta$ may be an important variable in AD pathogenesis. To explore this hypothesis, we produced affinity purified polyclonal antibodies to $A\beta$ 37–42 which, after β 40 column absorption, distinguish β 42 from β 40.

Peptides representing $A\beta$ 34–40 and 37–42 with an added N-terminal lysine were synthesized on an Ap-

plied Biosystems synthesizer using Fmoc chemistry, linked to carrier with glutaraldehyde and dialyzed against 0.1 M phosphate buffer, pH 7.0. Rabbit antisera were raised as previously described [3], affinity purified on peptide agarose bead columns, and tested against synthetic β 40 and β 42 (Dr. Nick Ling, Whittier Institute). Affinity purified antibodies to β 37–42 were absorbed on a β 40 column and 20 μ g/ml of aggregated β 40 to remove β -conformation dependent antibodies. The β 40 absorbed 37–42 antibody was used in subsequent studies ('anti-42'). The β 34–40 antisera from one rabbit ('anti-40') was predominantly reactive against β 40 and was specific for β 40 after absorption on β 42. Immunocytochemistry was performed on deparaffinized, formic acid treated 8 μ M sections from 5 AD cases ranging from 71 to 92 years and 3 age-matched controls using a Vectastain kit and diaminobenzidine as described elsewhere [3]. Dr. Z. Alton of BABCO (Richmond, CA) produced monoclonal antibody 10G4 to native β 40 which we have shown labels amyloid in AD and both β 40 and β 42 [17].

Immunoprecipitations from 10^7 cpm of 16 h conditioned media (CM) from 150 μ Ci TransS³⁵ labeled normal human fibroblasts or Chinese hamster ovary

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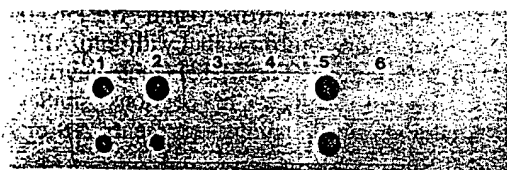


Fig. 1. Dot blot of 10 ng samples of peptides showing 1:500 and 1:1,000 dilutions of anti-42 against β 42 (columns 1 and 2), or β 40 (columns 3, 4), and anti-34-40 against β 40 (column 5), and β 42 (column 6).

cells overexpressing APP770 (CHO770, from Dr. S. Sisodia) were carried out as previously described [4].

On dot blots (Fig. 1), anti-42 recognized β 42 but not β 40 while the reverse was true for anti-40. On Western blots (Fig. 2A), in contrast to 10G4 anti-42 recognized β 42 but not β 40. Anti-40 failed to detect A β on Westerns. Immunoprecipitation of synthetic peptides followed by Western analysis for A β proved the specificity of the β 42 antibody for immunoprecipitation analysis. In Fig. 2B, we show anti-42 immunoprecipitates β 42, but fails to immunoprecipitate β 40. With difficult and expensive direct C-terminal sequencing there is a risk of inefficient recovery and an underestimation of the percentage of β 42 [5]. Having shown specificity in immunoprecipitates and optimized the recovery, we sought to detect soluble β 40 and β 42 in CM of labeled cells using anti-42 and affinity purified anti-34-40. In CM of both types of metabolically labeled cells, anti-42 brought down a fraction of the β 40 antibody's 4 kDa A β (Fig. 2C). As expected, APP transfected cells produced far more 3 and 4 kDa A β immunoreactive material. Scanning with an LKB Ultrascan densitometer indicated that anti-42 gave 16.6% of the fibroblast A β 40 4 kDa counts and 18.6% of the CHO770 A β 40 4 kDa counts, in rough agreement with a report using a sandwich ELISA for β 42 [16].

In sections from frontal cortex of 5 AD patients, β 42 specific antibodies stained both plaque cores and diffuse deposits (Fig. 3) and occasional neurons, chiefly large pyramidal cells. Vascular amyloid was stained by anti-42 (Fig. 3B), but more prominently by anti-40 (Fig. 3C,G) which labeled only a subset of plaques, but very intensely. Absorption with 20 μ g/ml of peptide antigen completely eliminated amyloid immunostaining (Fig. 3D-F). In contrast, absorption with β 42 failed to block anti-40 staining (Fig. 3G). The observation of robust anti-42 staining of diffuse plaques in cortex shows the β 42 C-terminus in one of the earliest A β deposits. Vessels also labeled in agreement with new sequence data [12], but anti-40 was comparatively selective for vessels (Fig. 3C).

In sharp contrast to the frontal cortex, diffuse deposits in the striatum which were readily labeled by 10G4 monoclonal (Fig. 3H) stained very weakly or not at all with anti-42 (Fig. 3I) or anti-40 (Fig. 3J). Rare anti-42 positive plaques were found, generally compact in nature, where many diffuse deposits were labeled by 10G4 monoclonal. While the more severe cases tended to have more amyloid in cortex and in the striatum, the reported phenomena were observed in all AD cases examined and normals with plaques. In agreement with other authors, despite the presence of many plaques in

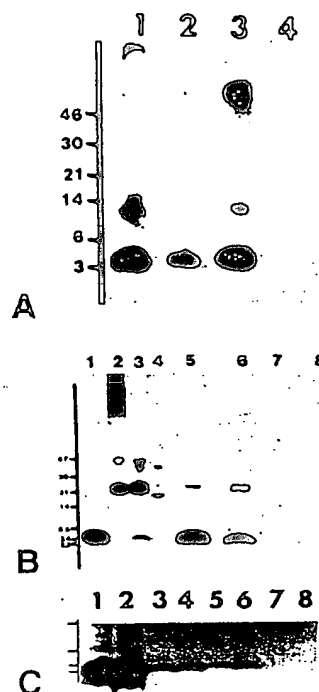


Fig. 2. A: Western analysis of 50 ng of β 42 (lanes 1 and 3) and 50 ng of β 40 (lanes 2 and 4) with monoclonal antibody 10G4 mapping to β 5-13 (lanes 1 and 2) vs. anti-42 (lanes 3 and 4). B: immunoprecipitates of 200 ng of β 40 or β 42 with different antibodies detected by Western analysis with anti-A β monoclonal 10G4 and anti-mouse IgG peroxidase. Lane 1 = 25 ng β 40 standard; lane 2 = 10G4 alone; lane 3 = 10G4 with β 40; lane 4 = anti-42 with β 40; lanes 5,6 = anti-42 with β 42; lanes 7,8 = anti-42 absorbed with 10 μ g 37-42 peptide. C: immunoprecipitates of CM from CHO770 cells or normal human fibroblasts labelled for 16 h in 1% dialyzed fetal calf serum and cysteine and methionine free medium with 300 μ Ci of TransS label 35 S (ICN, Costa Mesa, CA). Labelled media were cleared, incubated with 10 μ l of antibody/ml media, and immunoprecipitates were brought down and run out on 10-20% Tris Tricine gels and autoradiographed. Mol.wts. as in B. Lanes 1, 2, 5 and 6 = CHO770. Lanes 3, 4, 7 and 8 = fibroblasts. Lanes 1-4 = affinity purified 34-40; lanes 5-8 = anti-42.

the AD dorsal striatum, only these rare compact deposits were positive for traditional amyloid stains like thioflavin S [1] and associated with fibrillar amyloid and abnormal neurites [6]. These observations are intriguing because the striatum is not associated with many neuritic plaques or significant clinical or pathological evidence of synaptic or neuronal damage [15]. We are currently investigating the possibility that region specific $A\beta$ metabolism results in C-terminal truncated forms of $A\beta$ which don't readily form amyloid fibrils and are not associated with neurodegeneration.

Our anti-42 antibodies also detected selected neurons, most commonly large pyramidal cells, in AD but also normal frontal cortex (Fig. 4A). This neuronal staining contrasted sharply with the more prevalent labeling of both diffuse and mature plaques (Fig. 4B) and more closely resembled labeling by APP antibodies

as shown with affinity purified anti-APP176-186 in Fig. 4C. Many more neurons label with anti-APP (Fig. 4D) than with anti-42 (Fig. 3B). Plaque neurites also labeled with anti-APP as in previous studies [3]. Whether the neuronal staining represents $A\beta$ or APP remains unclear.

APP717 mutations migrate with early onset AD and cells expressing them produce normal amounts of total $A\beta$, but substantially more $\beta 42$ [12]. Our data show that in the cortex, the majority of diffuse and other deposits are strongly positive for $A\beta 42$ while a subset of deposits are positive for $\beta 40$ in agreement with a recent report [8]. In contrast to the cortex, in the striatum, neither the anti-42 or anti-40 show strong staining relative to more N-terminal $A\beta$ antibodies or to staining in cortex. Collectively, these data imply that the length of $A\beta$ is an important factor regulating

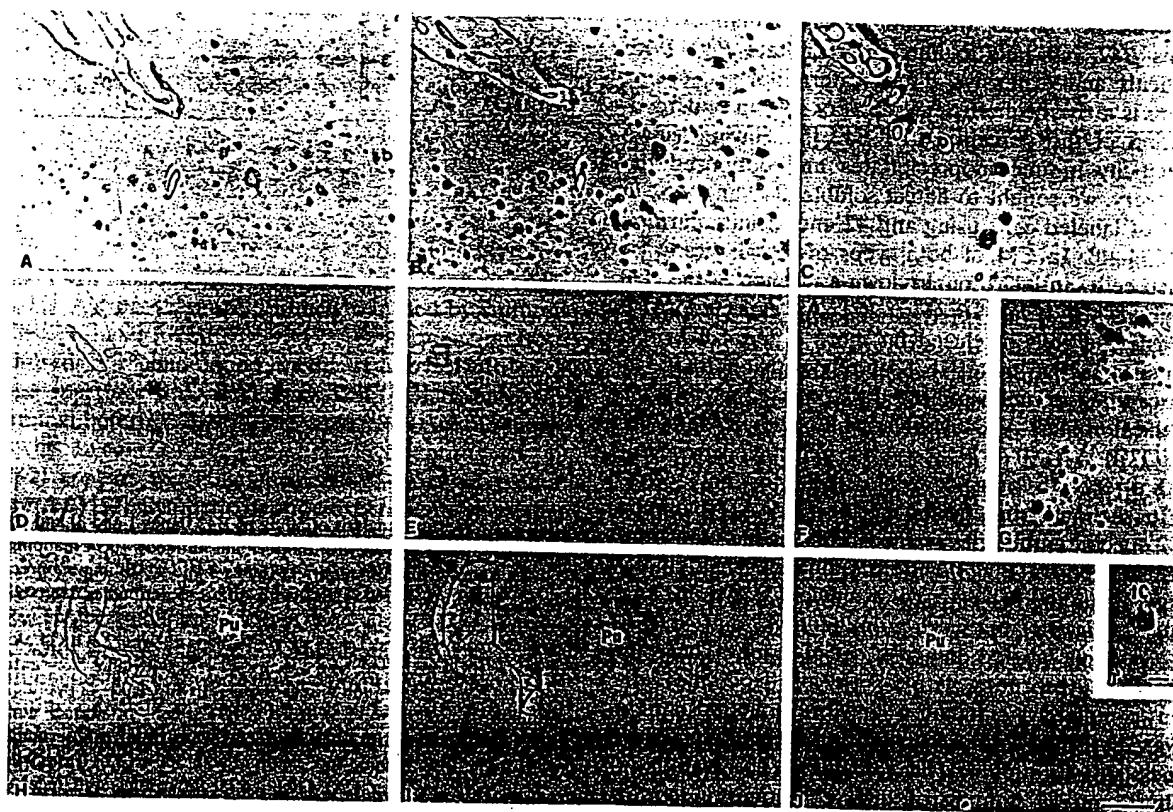


Fig. 3. A-G: AD frontal cortex immunostained with: (A) 10G; (B) 10G4 anti-42 monoclonal; (C) anti-40; (D) 10G4 absorbed $\beta 1-40$; (E) anti-42 absorbed $\beta 37-42$; (F) anti-40 absorbed $\beta 34-40$; (G) anti-40 absorbed $\beta 1-42$; (H-J) adjacent sections of putamen (Pu) with (H) 10G4, (I) anti-42, (J) anti-40. A-J: original magn. $40\times$. Bar = $250\ \mu\text{m}$. Inset J': anti-40 on insular cortex (IC) in same section as (J). Orig. magn. $100\times$. Bar = $5\ \mu\text{m}$.

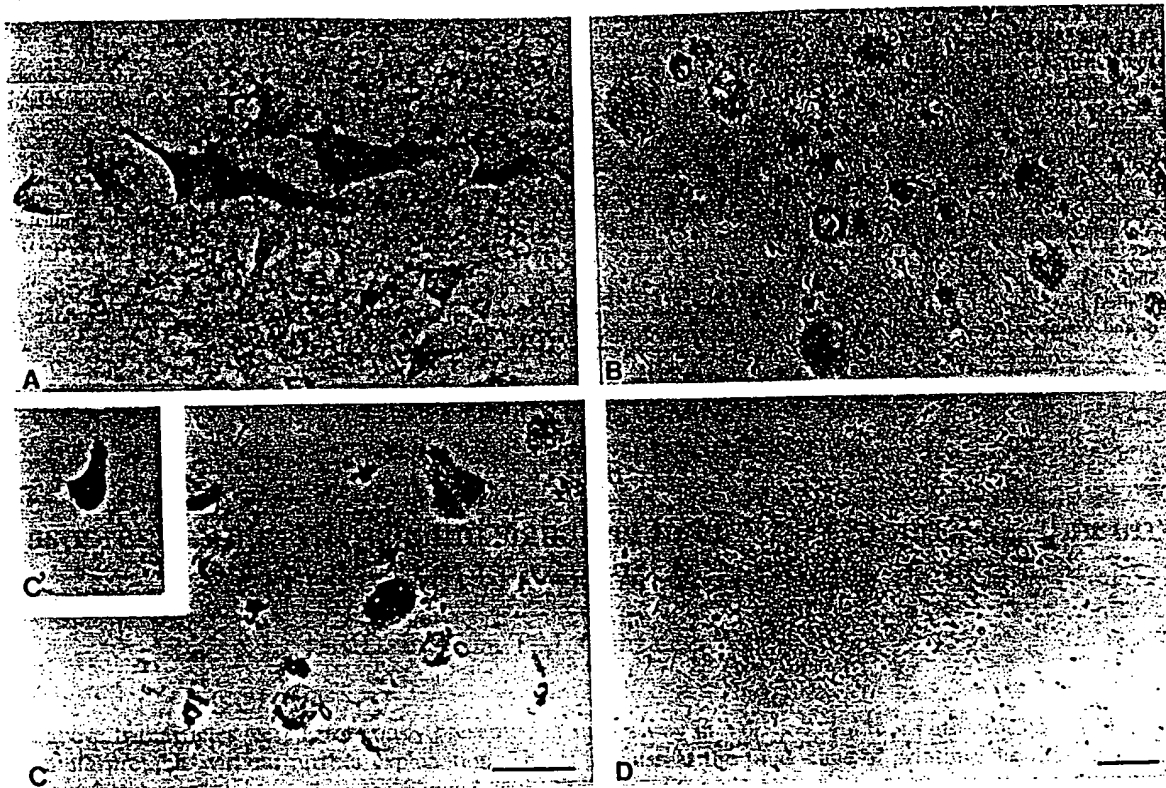


Fig. 4. A: anti-42 labeling some large pyramidal neurons in normal cortex. B: anti-42 labeling diffuse and cored plaques in AD brain. Similarly, anti-APP176–186 labeled neurons in normal (C; bar = 25 μ m) and in AD (D; bar = 100 μ m) cortex. B,D: orig. magn. 100 \times ; A,C: orig. magn. 400 \times .

β -amyloidosis. Our data also suggest region dependent variation in A β deposits which may be related to plaque progression.

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